Microtubules, Structure Dynamics, and Associated Proteins

2250-Pos Board B387

Transformation of Taxol-Stabilized Microtubules into Inverted Tubulin Tubules Triggered by a Tubulin Conformation Switch

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Bundles of taxol-stabilized microtubules (MTs) - hollow protein nanotubes comprised of assembled αβ-tubulin heterodimers - spontaneously assemble above a critical concentration of tetravalent spermine and are stable over long times at room temperature. Here we report that at concentrations of spermine several-fold higher the MT bundles (B_{MT}) quickly become unstable and undergo a shape transformation to bundles of inverted tubulin tubules (B_{ITT}), the outside surface of which corresponds to the inner surface of the B_{MT} tubules. Using transmission electron microscopy and synchrotron small-angle X-ray scattering, we quantitatively determined both the nature of the B_{MT}-to-B_{ITT} transformation pathway and the structure of the B_{ITT} phase. Inverted tubulin tubules provide a platform for studies requiring exposure and availability of the inside, luminal surface of MTs to MT-targeted drugs and MT-associated proteins. (ref. *Nature Materials 2014, **13**, 195-203*)

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Single-Molecule FRET Reveals an Extended Structure of Tau Bound to Tubulin Heterodimers

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Tau is an abundant neuronal protein that promotes tubulin polymerization and stabilizes microtubules (MTs) in the axons of neurons. The disruption of its normal biological function as well as its aggregation and deposition into neurofibrillary tangles has been proposed to contribute to several neurodegenerative disorders known as tauopathies, including Alzheimer's disease. Although taús interaction with MTs has been extensively studied, a detailed structural characterization of the complex is lacking. This deficit is, in part, due to the fact that tau is intrinsically disordered in solution and remains largely so upon binding to MTs, making its structural characterization challenging. Here, we identified conformational changes in tau underlying its binding to tubulin. To do so, we used intramolecular single-molecule Förster Resonance Energy Transfer (smFRET) measurements of the full-length tau protein upon binding to soluble tubulin heterodimers under non-assembly conditions. Tau was labeled with donor and acceptor fluorophores at multiple pairs of positions to cover all domains of interest. We observed that tau undergoes significant structural rearrangements when binding to tubulin. Specifically, it results in a loss of long-range contacts between the two termini and the microtubule binding region (MTBR) that characterizes taús compact solution conformation, leading to an overall expansion of its structure. Moreover, we contrast the structures of two isoforms of tau which differ in the number of MTBR (2N3R and 2N4R). From this work, we propose a topological model of the structure of tau bound to tubulin, which provides insight to the molecular mechanism of tau-mediated tubulin polymerization into MTs.

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Statistical Mechanics Provides Novel Insights into Microtubule Stability and Mechanism of Shrinkage

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Microtubules are nano-machines that grow and shrink stochastically making use of the coupling between chemical kinetics and mechanics of its constituent protofilaments (PFs). Understanding this coupling is crucial for understanding the stability and dynamics of the tubulin assembly, and for predicting multi-scale effects induced by a number of regulating agents. In this work we investigate the stability and shrinkage of microtubules taking into account inter-protofilament interactions and bending interactions of intrinsically curved PFs. Computing the free energy as a function of PF tip position, we show that the competition between curvature energy, inter-PF interaction energy and entropy leads to a rich landscape with a series of minima that repeat over a length-scale determined by the intrinsic curvature. Computing Langevin dynamics of the tip through the landscape and accounting for depolymerization, we calculate the average unzippering and shrinkage velocities of GDP protofilaments and compare them with the experimentally known results. Our analysis predicts that the strength of the inter-PF interaction (E^s_m) has to be comparable to the strength of the curvature energy (E^b_m) such that $E^s_m - E^b_m \approx 1k_BT$, and questions the prevalent notion that unzippering results from the domination of bending energy of curved GDP PFs. Our work demonstrates how the shape of the free energy landscape is crucial in explaining the mechanism of MT shrinkage where the unzippered PFs will fluctuate in a set of partially peeled off states and subunit dissociation will reduce the length.

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Molecular Dynamics Study of the Effect of Polyamine on Microtubule Conformations

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Microtubules (MTs) play important roles in cell division, intracellular protein transport, and cell structure and movement in eukaryotic cells. MTs are hollow supramolecular polymers made of alpha and beta tubulin heterodimers. Microtubule assembly in cells require a proper orientation of tubulin molecules to the MTs end. Experiments have shown that polyamines such as spermine can facilitate the diffusion of tubulin dimers in the process of nuclei growth or MT growth. The mechanism of this aggregation is the bridging interaction of polyamines with highly negative C-terminal tail of tubulin surface which helps tubulin together. Recent experiments also show that with a high concentration of spermine, bundles of taxol-stabilized MTs undergo a shape transformation to bundles of inverted tubulin tubules. However, the molecular mechanism underlying the transformation is unclear. We performed all atom molecular dynamics simulation of tubulin clusters in presence of spermine in an explicit water solvent to study the structural dynamics of alpha/beta tubulin and microtubule protofilament. We discuss our results on the interaction between spermine and tubulin proteins and the effect of spermine on conformational dynamics of MT protofilaments.

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Molecular Basis for Age-Dependent Microtubule Acetylation

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Microtubules are decorated with chemically diverse and evolutionarily conserved posttranslational modifications that mark them for specialized functions in cells. Other than viruses and clathrin cages, microtubules are the only known hollow polymers in eukaryotic cells. Acetylation on alpha-tubulin Lys40 stands out as the only known tubulin posttranslational modification located in the microtubule lumen. It marks stable, long-lived microtubules and is required for polarity establishment and directional migration. Tubulin acetyltransferase (TAT) presents a puzzle: its activity is stimulated by microtubules, yet the luminal location of Lys40 hinders its access. TAT was proposed to access its substrate by diffusing from microtubule ends, engaging the Lys40 loop from the exterior of the microtubule or gaining access through openings or defects in the microtubule. To elucidate substrate access by TAT and uncover the molecular basis for the *in vivo* correlation between acetylation and microtubule age, we combined X-ray crystallography, electron microscopy, biochemical and single-molecule fluorescence analyses, and modeling. We report the first crystal structure of TAT in complex with a bisubstrate analog that together with structure-based functional analyses constrains the active enzyme to the microtubule lumen and reveal the structural determinants for Lys40 loop recognition. Unexpectedly, acetylation by TAT proceeds stochastically along the microtubule without a preference for ends despite the luminal location of the acetylation site. Single-molecule fluorescence imaging reveals that TAT efficiently and stochastically scans the microtubule, while modeling and kinetic analyses demonstrate that TAT catalytic activity, rather than diffusion through the lumen, is rate-limiting. Consistent with this, our 1.35Å X-ray complex crystal structure reveals that the TAT active site is suboptimal for supporting the proton transfer required for acetylation. Thus, by virtue of its preference for the microtubule and its low catalytic rate, TAT alone can function as an enzymatic timer for microtubule lifetimes.